Biosynthesis of an Anti-biofouling Surface Binding Polymer using Orthogonal Translation

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Fouling of surfaces is an issue in both the medical and naval industry. According to Shirtliff & Leid, 2009, 60% of infections associated with hospitals are due to biofilm formation. Furthermore, a 2011 study conducted by the Woods Hole Oceanic Institution states that biofilm formation causes increased frictional drag time in ships, directly costing the US Navy about $200 million per year and lowering the life spans of ships.

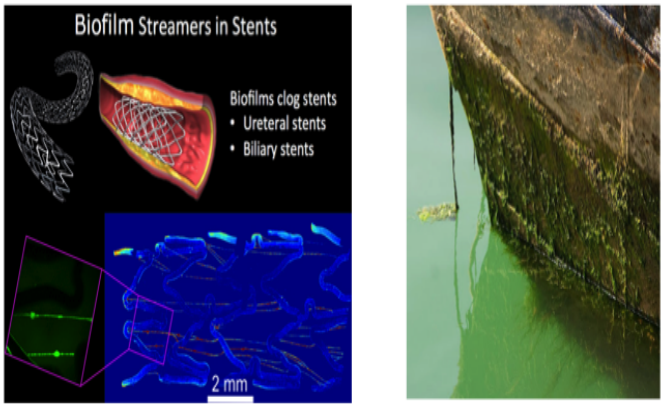

Figure 1. Biofilm formation on medical implants and ships.

Simultaneously, development of this anti-biofouling peptide requires stringent regulations. However, these peptides will be potentially toxic to the GRO that they are made in, so it is first necessary to develop a tightly controlled expression system. In this way, we are improving the expression system to produce toxic proteins and, in the process, developing anti-biofouling peptides.

We developed the T7 Riboregulation Expression System to carry out the synthesis of the anti-biofouling peptide as well to develop an improved T7 expression system that is robust and reusable (in terms of portability between E. Coli). Though ubiquitous, the current BL21 (DE3) strain of *E.coli* for T7 expression is leaky due to the weak suppressing promoter lacUV5 that drives T7 RNA

polymerase in the DE3 strains (Samuelson, 2011). As a result, low levels of toxic protein is constitutively expressed, ultimately killing the host it was made in and in turn lowering the overall yield of the protein produced. Our goal is to reduce the background expression of T7 RNA polymerase and create a tightly controlled expression system for heterologous, toxic proteins in *E. coli*. Since the anti-fouling peptide will only be expressed when we specifically induce the expression, we will most likely have a higher yield than if we used the current BL21(DE3) strain. This controlled expression is possible because the T7 Riboregulation System has locks in place that prevent the expression of T7 RNA Polymerase which will then drive the transcription and then translation of the gene of interest, view Figure 2 (Isaacs, Dwyer *et al*., 2004).

In parallel, we also designed an anti-biofouling peptide (Figure 2b). There are two components to this peptide: the mussel adhesion protein (MAP) anchoring domain and the antimicrobial peptide (AMP) domain. We focused on mussel foot proteins for the anchoring domain, using a combination of *Mytilus galloprovincialis* Foot Protein type 5 (Mgfp-5) and *Mytilus Edulis* Foot Protein (Mefp-1) (Lee *et al.*, 2008). These proteins are able to attach to surfaces using L-Dopamine (L-DOPA), which can bind to surfaces. An integral part of developing this peptide is the co-translational insertion of L-DOPA into our peptide, which is more efficient than the current post-translational method for producing DOPA-containing peptides.

For the antimicrobial domain, we chose to focus on LL-37. LL-37 is comprised of anionic and zwitterionic bilayers, which are important anti-fouling

traits. LL-37 acts on the surfaces of cells, forming a toroidal pore that pierces through the cells of biofilm strains. Transcriptomtic and biochemical investigations have shown that LL-37 can act against the common biofilm strain *P. aureginosa* and prevent uncontrolled growth of microbes (Nagant *et al.*, 2012). Previous research has successfully conjugated LL-37 to a carbohydrate-binding module from *Clostridium thermocellum*, and has successfully shown LL-37 functionality in the conjugated state (Ramos et al., 2010).

We also plan to develop assays to test out the strength of the peptide's adhesion using both an erosion rig simulation and atomic force microscopy analysis. The erosion rig is unique in that it introduces coating to the media and simulates drag through liquid. We will also examine the ability of the anti-biofouling peptide to inhibit biofilm formation, using either the Crystal Violet Staining protocol or the MBEC Assay, supplemented with confocal microscopy.

In conclusion, we have developed an improved T7 Expression system that can be used to express toxic proteins, which will hopefully advance the expression of synthetic or toxic proteins within *E. coli*. Furthermore, with this novel expression system, we will have developed a viable anti-biofouling peptide as a possible solution to prevent biofilm growth.

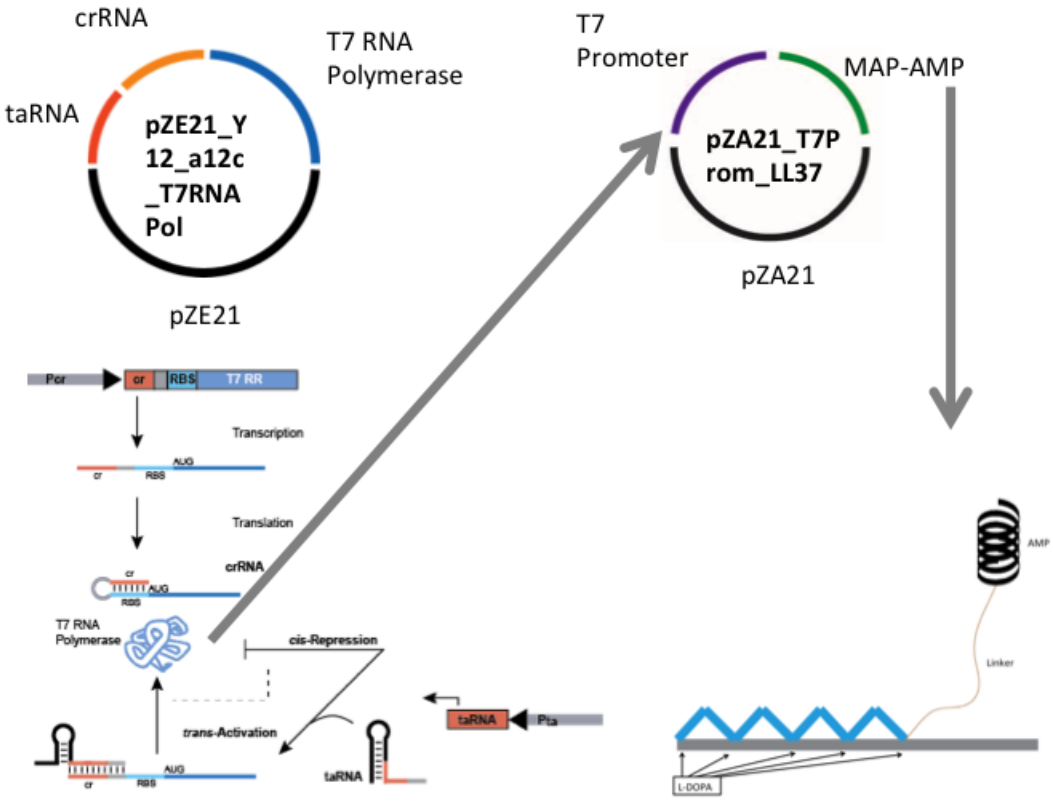

Figure 2. Artifical Riboregulatory System to produce controlled expression of the anti-biofouling peptide (MAP-AMP). MAP-AMP expression depends on T7 RNA Polymerase, which in turn is repressed at the transcriptional and translational levels unless both ATC and IPTG are present. crRNA= *cis* repressing RNA. taRNA= *trans* activating RNA. MAP= Mussel Adhesion Protein. AMP= Antimicrobial Peptide (LL-37). P_{cr} = promoter that expresses stem loop formed at 5' end of mRNA. P_{ta} = promoter that expresses small, noncoding RNA that targets crRNA with high specificity. RBS= Ribosomal Binding Site.

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